Diagnostic and Epidemiologic Study of Human Brucellosis at Al-Najaf province,

دراسة تشخيصيه و وبائية للحمى المتموجه في محافظة النجف الاشرف

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الخلاصة:

خلفية البحث: حمى مالطه هي واحدة من الأمراض المهنية الخطرة لكل من منتجي الألبان ، العاملين مع الماشية وفي المسلخ والأطباء البيطريين و العاملين في المختبرات. وترافق الاصابة مجموعة كبيرة من الأعراض السريرية التي تتطلب استخدام اختبارات تشخيصية فعالة .ان اختيار طريقة التشخيص الوبائية وحسب المنطقة واتخاذ القرار بالعدوى يعتمد على حجم الإصابة ، المعلوماتِ السريريةِ المقدمة من قبل المريض التي يجب ان تتضمن الرقعة الجغرافية ، نوع العمل وان كان مسافر الى المناطق الريفية او الى دولة موبوءة بهذا المرض , هل أحد افراد العائلة مصاب بالمرض , العلاج الذي يأخذه المريض ماهو تأثيره الإيجابي والسلبي على المريض. تميل جرثومة حمى مالطا الى الانسجة الغنية بالأوعية الدموية .

الهدف: تهدف الدراسة إلى تقييم الجوانب الوبائية للسكان وفقا لنوع الجنس و العمر. ودراسة خاصية و حساسية اختبارين، الأول هو استخدام الاختبار التقليدي (روز البنغال) ، والتقنية الجزيئية الحديثة (RT- PCR) ومقارنة النتائج بينهما.

المنهجية: نوع الدراسة هي دراسة تشخيصية تتضمن 41 عينةِ دمّ من المرضى المشكوك بإصابتهم بحمى مالطا، مِنْ كلا الجنسين، أعمارهم كَانتْ بين 14- 45 سنة . المرضى كَانوا يأتون الى محافظةِ النجف مِنْ المناطق الريفية البعيدةِ المحيطة بالمحافظة ومن مركز المحافظة من عيادات خاصة للمدة من كانون الثَّاني إلى كانون الأولِ 2015. والتقييم المصلي لعدوى حمى مالطا تم باستعمال طريقةِ الاختبار المصلي وتقنية الفحص الجزيئي.

النتائج: بينت نتائج الدراسة بان المجموعة العُمرية الأكثر تكراراً كَانتْ أكثر مِنْ 35 سنة (29.3) وان (58.54 %) من العينة هم من الريف، وان متغيّرات تنبؤيه كَانتْ مسؤولة عن العينة لحمى مالطا , أثناء سَنَةِ واحدة لوحظ قيمةَ تنبؤيه إيجابيةً =50%، قيمة تنبؤيه سلبية =95.2% و دقة =73.1 % مِنْ التشخيص المصلي قورن بالتشخيص الجزيئي الدقيق للمرضى 41 مريض. الحسّاسية العالية (90.9 %) لَها كشفُ لحمى مالطا بالفحص الجزيئي الدَقيق ،والمصلي =66.7%.

الاستنتاج: تقدر الاصابة في المناطقِ الأكثر إصابة (المناطق الريفية) أقل عند مقارنةَ عدد سكانها من الاقل عمراِ; كما ان الأمان الطبي مهمُ جداً في المختبرات المستخدمة في تشخيص المرض صنفت منظمة ال CDC العراق من المناطق عالية الاصابة بحمى مالطا .

التوصيات: تفادي منتجات الحليب غير المعقمة والحليب الطازج غير المغلي , ويجب ان تكون المختبرات السريرية عند عزل المسبب المرضي تحت شروط الأمان الصحي .والعلاج المبكر للمرض عند ظهور العلامات السريرية يقضي على المرض ويقي من مضاعفاته المزمنة.

Abstract

Background: Maltese fever is one of the dangerous occupational diseases for dairy producers, processor and staff cattle slaughterhouse, veterinarians and laboratory personnel. Infection accompanies a wide range of clinical symptoms that require the use of effective diagnostic tests. The choice of the epidemiological diagnosis in the region and the decision of the infection depends on the size of the infestation., Clinical information provided by the patient must include the geographical area, work type Was traveling to rural areas or affected countries this disease Is a family member with the disease, treatment he takes the patient what is positive and negative impact on the patient. Germ Malta fever tend to tissue rich in blood vessels

Aims of study: The study aims to evaluate the epidemiological aspects of the population according to gender and age. And the study of the property and the sensitivity of the two tests, the first is the use of a conventional test (Rose Bengal), and modern molecular technique (RT- PCR) and compare the two results.

Methodology: Type of study is a diagnostic study includes 41 blood samples from patients with suspected Malta fever, from both gender diagnosed questionable, the ages were between 14-45 years. Patients were attending to the province of Al-Najaf Al-Ashraf from rural area surrounding the governorate and the center of the province, and attend private clinic from January to December 2015 in order to evaluate serum infection of Malta carried out using serological testing and technology of molecular test method

Results: A total of 41 patients with suspected brucellosis had been included in this study. The most frequent age group was more than 35(29.3%) years. The percentage to an urban cases were (58.54%) and the percent of rural area**.** Brucellosis, variations during one year shows positive predictive value=50%, Negative

predictive value=95.2% and Accuracy =73.1 % from diagnosis of serological compared to Real time PCR proportion of 41 patients. High sensitivity (90.9 %) has detection of Brucellosis by Real Time PCR, and the Specificity=66.7%.

Conclusion: Estimated incidence in most regions injury (rural areas) may be less compared to the younger population. Medical safety is very important in the laboratory. Organization of the CDC classifies Iraq from high incidence areas with Malta fever.

Recommendations: Avoid unpasteurized hand milk products and fresh milk which is not boiled. Clinical laboratories must be under the terms of health safety when isolate the pathogen. And early treatment of the disease at the onset of clinical signs of the disease eliminates protect against chronic doubled.

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INTRODUCTION

Brucellosis is the commonest human zoonotic disease. It is caused by Brucella species: Gram- negative, aerobic, facultative intracellular coccobacilli bacteria. It is transmitted to human through consumption of unpasteurized dairy products or undercooked meat from infected animals and direct contact with infected animals [(1)], it can enter the body via skin wounds, mucous membranes, or inhalation the bacteria that causes brucellosis may also lead to infection. This risk is generally greater for people in laboratories that work with the bacteria. Person-to-person transmission is rare, infected mothers who are breast-feeding may transmit the infection to their infants. Sexual transmission, tissue transplantation or blood transfusions have been rarely reported[(2)]. Initial symptoms can include: Fever, sweats, malaise, anorexia, headache, pain in muscles, joint, back fatigue and spontaneous abortion in pregnant woman. Some signs and symptoms may persist for longer periods of tissue, that may continue to trouble patients for as long as 25 years [(3)].

These High-risk regions include the South and Central America, Eastern Europe, Asia, Africa, and the Middle East. In these areas[(4)] . brucellosis is primarily enzootic in cattle, sheep, and goat populations, who is exposed to the bacteria that cause brucellosis is at risk for infection [(5)].Interest in brucellosis has been increasing because of the growing phenomena of international tourism and migration, in addition to the potential use of Brucella as a biologic weapon[(6)].

Diagnosis of brucellosis is complicated because the disease may have an incubation period varying from five days to five months and can progress in various forms: acute, chronic or asymptomatic [(7)]. Symptoms of the acute phase of brucellosis fever and weakness in humans is common to a wide range of different diseases. Therefore, the final diagnosis of brucellosis has to be obligatorily confirmed by laboratory testing. Brucellosis diagnostics is based on bacteriological and molecular methods (direct tests), and serological in vitro and in vivo methods (indirect tests) [(8)]. The choice of the diagnostic method depends on the overall epidemiological situation in the region and the objectives of the study: validation of the diagnosis, screening (monitoring), cross-sectional studies or confirmation of brucellosis-free status of the region [(9)]. Rose Bengal is a broadly used simple method of brucellosis diagnostics is the test). It is a simple spot agglutination test where drops of stained antigen and serum are mixed on a plate and any resulting agglutination signifies a positive reaction. The results are received in several minutes[(10)] .

Molecular biological techniques, often based on the polymerase chain reaction (PCR) amplification, are successfully used for Brucella identification and typing. The first crucial step of PCR based methods is DNA isolation from biological samples [(11)]. The most simple and reliable method of Brucella identification is PCR with a single pair of primers, specific to the bacterial DNA sequences, such as 16S - 23S rRNA operon, IS711 or BCSP31 genes [(12)].

Bosphore, Real Time- PCR Brucella detection kit detects Brucella spp. DNA in human biological sample .The analytic sensitivity is 7.5*102 copies /ml. Amplification and fluorescence detection as a novel approach to assess the molecular response to various infectious diseases [(13)].

OBJECTIVE

The study aims to evaluate the epidemiological aspects of the population according to gender and age. And the study of the property and the sensitivity of the two tests, the first is the use of a conventional test (Rose Bengal), and modern molecular technique (RT- PCR) and compare the two results.

MATERIALS AND METHODS

Ethics statement :Human Brucellosis case data were extracted from same patients by present and past case history. The study was approved by Research and Development department of faculty of medicine.

Study design and Patients: Sample size of diagnostic study was from 41 patients blood samples, from both genders with suspected Brucellosis according to professional clinical assessment, their ages were between 14-45 years. Patients were attending in Al- Najaf province from rural area and urban area, and patients attended private clinics between January and December from 2015.

Collection of samples: Five milliliters (ml) of venous blood were drawn from each patient by venipuncture (5ml disposable syringe). Blood was divided into 2 groups.

1. 3 ml of blood were collected in sterile gel serum tube for using Rose Bengal test and left for serum collection, which was stored at-20 C°.

2. 1 ml of blood were collected in EDTA tube and extraction of DNA from blood was done in each sample in the Eppendorf tubes (1-2) ml and were done immediately for RT- PCR test.

Serological Diagnosis

A-Agglutination for slide and tube tests Qualitative and Quantities Determination of Serum Antibody. Complete Agglutination Kits were used. This kit was supplied by (Biorex Diagnostic Company / United Kingdom) which included:

1. Agglutination reaction for Qualitative and quantities detection of **Brucellaabortus** in human was measured by agglutination technique using Kit.
2. Agglutination reaction for Qualitative and quantities detection of **Brucellamelitensis** in human was measured by agglutination technique using Kit.

Molecular Diagnosis

A- Extraction and Estimation.

1. DNA Extraction: DNA extraction kit was supplied by Bosphore Anatoligene works Company (Istanbul -Turkey), DNA extraction was performed according to the manufacturer s instructions.

B- Real Time PCR

1-Real – Time PCR test for qualitative detection of Brucella in human was measured by RT-PCR Kit (Bosphore Anatoli Biotechnologies –company –Turkey) .Programming the Real-Time PCR Thermo cycler conditions (Amplification):Real-Time PCR Thermo cycler conditions were set according to kit instructions .

RESULTS :

1. Distribution of age

Table (1): Distribution of age with positive Brucellain the study sample (n=41) .

Age group	Frequency	Percent
<=20	7	17.1
21-25	9	22.0
26-30	8	19.5
31-35	5	12.2
>35	12	29.3
Total	41	100.0

A total of 41 patients with suspected brucellosis had been included in this study. The age of patients shows in table 1. The most frequent age group was more than 35(29.3%) years; the second most frequent age group was 21-25 years (22.0%).

2-Residence distribution

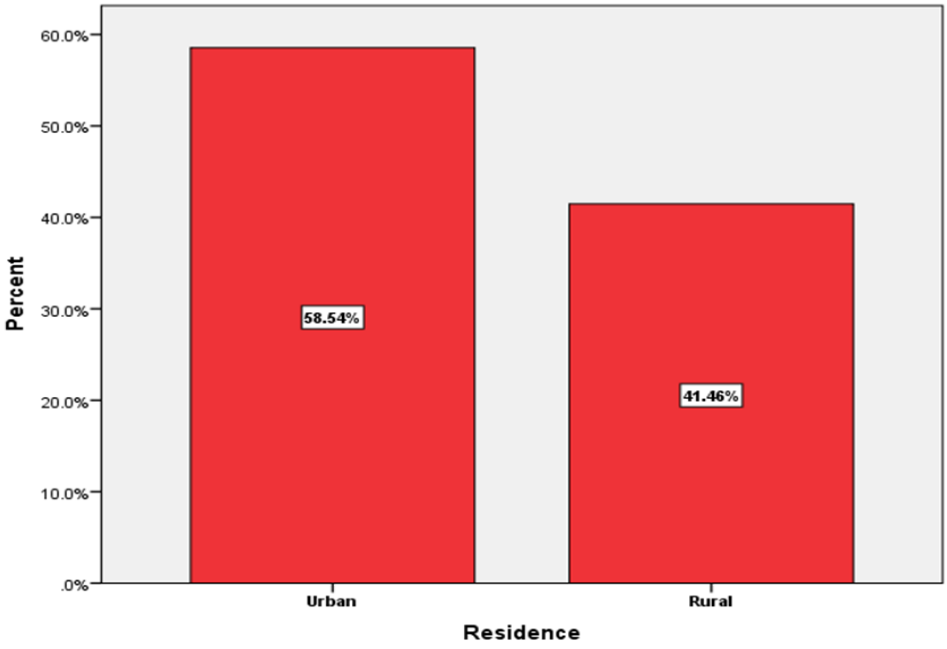

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Figure (2): Residence of studied patients.

This figure showed the human Brucellosis cases at the rural and urban from 2015, and compared between them (Figure: 2). the percentage to an urban cases were 58.54% and the percent of rural were 41.44% determine statistically significant differences between each from the rural area and urban.

3-Gender distribution

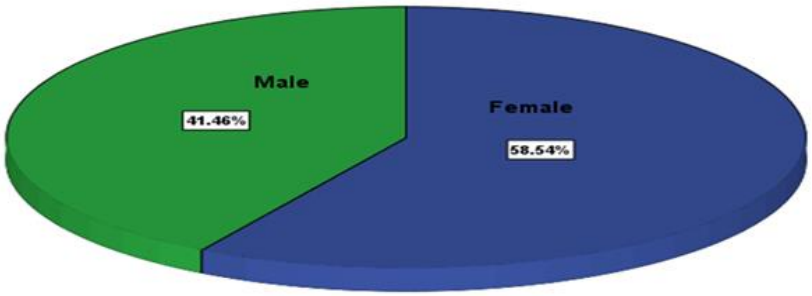

Figure 2: Gender distribution of study sample

In this figure (figure: 2) showed the difference of gender was the most frequent at female was about (58.54%).

MOLECULARRESULT

Table (2) Diagnostic accuracy of serology compared to Real time PCR proportion of patients (n=41).

		Real time PCR positive	Negative	Total
Serology	**Positive**	10	10	20
		90.9%	33.3%	48.8%
	Negative	1	20	21
		9.1%	66.7%	51.2%
Total		11	30	41
		100.0%	100.0%	100.0%

In this table(Table 2) showed , Predictive variables have been responsible for 41 patients Brucellosis, variations during one year showed positive predictive value=50%, Negative predictive value=95.2% and Accuracy =73.1 % from diagnosis of serological compared to Real time PCR proportion of 41 patients. High sensitivity (90.9 %) has detection of Brucellosis by Real Time PCR, and the Specificity=66.7% .

DISCUSSION

High sensitivity (90.9 %) has detection of Brucellosis by Real Time PCR, and the Specificity=66.7% .Serology test of human Brucellosis must take into checking individuals develop antibodies but do not become infected, that agreement with **Chen, et al., (2013) [(14)].**Serological test

result and presence of clinical signs with brucellosis are essential to screening, specificity was reduced to 90.9 %that nearby Gómez MC,et al(2008)[15], positive Real Time PCR results were specificity to 66.7% [16].

Residence of patients was conducted of human Brucellosis, the rural area (41.44%) was considered an appropriate for the study of human Brucellosis due to its provision of a suitable environment for animals .In this study found an urban area (58.54%) was more than rural due to milk production unsterile ,due to colonized of bacteria leading to frequent milk shedding [17]. In urban, consumption of home-made milk products is a risk factor for brucellosis infections. Sero-positivity for brucellosis and age, sex, and the consumption of fresh cheese and cream made from unpasteurized milk [18].Important role in the types of vegetation that can grow in certain areas, temperature ranges, and other environmental conditions important to the survival and transmission of Brucella[19] .

The most frequent at female was about (58.54%), that disagreement with larger numbers of reported human cases during 2008 occurred predominantly in a specific gender (male, 70.2%), andthe most frequent age group was more than 35(29.3%) years, that agreement with age range (30–59 years old, 64.7%)[20].

CONCLUSION:

1. Estimated incident in higher areas (rural) may be compared by the younger population.
2. Medical safety is very important in the laboratory.
3. Organization The CDC classifies Iraq from high incidence areas.

RECOMMENDATIONS:

1. Avoid unpasteurized milk and fresh milk products is boiled.
2. Clinical laboratory, when isolated pathogen must be under the terms of health safety.
3. Early treatment of the disease when the onset of clinical signs that eliminates the disease.

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